FATTY ACIDS PROFILE OF MEAT, MUCOSAL sIgA CONCENTRATION AND PRODUCTION INDEX OF BROILER AS A RESPONSE TO CHLORELLA sp. ADMINISTRATION IN THE DIET

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The front cover illustrates the sketch of leaves and seeds of legume and grass forming a buffalo's horn (designed by Agung Purnomoadi)

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[*DAFTAR ISI*]

Jurnal Pengembangan Peternakan Tropis [JPPT-ISSN 0410-6320] terakreditasi No. 65a//DIKTI/Kep/2008 dan memperoleh Hibah Pengintemasionalan Jurnal No. 016/SP.SIP/DP2M/VIII/2009 oleh Direktorat Jenderal Pendidikan Tinggi - Depatemen Pendidikan Nasional. Jurnal ini diterbitkan 4 kali dalam setahun, yaitu pada bulan Maret, Juni, September, dan Desember. Redaksi menerima tulisan/karya ilmiah hasil penelitian bidang peternakan yang belum pernah dipubilkasikan atau tidak sedang dipertimbangkan untuk dipublikasikan di jurnal lain. Biaya langganan per tahun adalah Rp. 250.000,- termasuk ongkos kirim [untuk luar pulau Jawa dan luar negeri dengan tambahan ongkos kirim].

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FATTY ACIDS PROFILE OF MEAT, MUCOSAL sIgA CONCENTRATION AND PRODUCTION INDEX OF BROILER AS A RESPONSE TO *CHLORELLA* sp. ADMINISTRATION IN THE DIET

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ABSTRACT

This study was carried out to investigate the effect of different levels of *Chlorella* sp. in the form of meal administrated in the diet of broilers throughout their life upon FA profile of the breast muscle, mucosal secretory IgA (sIgA) concentration and production index (PI) of broiler. Allotted in 18 pens, the total of 90 Ross day old chicks were assigned in completely randomized design by 3 of dietary treatments (T1: control (basal diet without enrichment of *Chlorella* sp.); T2: basal diet enriched with 5-g *Chlorella* sp./kg feed; T3): basal diet enriched with 10-g *Chlorella* sp./kg feed. At d-36, skinless breast meat was collected for FA determination. Practical standard of vaccination was performed to activate antibodies production and at d-36 gut mucosa was collected for sIgA analysis. *Chlorella* sp. administration had no significant effect (P>0.05) on the FA profile of breast muscle, mucosal sIgA concentration and producton index of broiler. In conclusion, the level and ratio between n-3 to n-6 polyunsaturated fatty acids (PUFA) as well as the nature of dietary PUFA source determine tissue PUFA composition. Beside through eicosanoid, *Chlorella* sp. may affect mucosal sIgA production through cytokines mediated effect. Although *Chlorella* sp. administration does not improve the production index of broiler, this treatment may produce broiler meat with lower fat content.

Keywords: broiler, Chlorella sp., fat, fatty acid, immunoglobulin, level, production

INTRODUCTION

Nowadays, consumer awareness upon the health benefit of n-3 polyunsaturated fatty acids (PUFA) is growing (Zuidhof *et al.*, 2009). At the same time, poultry meat becomes an important source of animal food protein. In monogastric animals it is well established that fatty acids (FA) profile of feed directly affects to the FA composition of fat depots (Rymer and Givens, 2005). Therefore, poultry meat may offer a potential alternate route for increasing dietary n-3 PUFA consumption. However, the fact that fast growth in broiler is accompanied by excessive fatness (Yu and Robinson, 1992), in which fat is considered unhealthy for the consumers (Wood *et al.*, 2008), may lower the consumer demand for enriched food product derived from broiler. Administration of *Chlorella* sp. in the diet could be one of the alternatives to enrich broiler meat with n-3 PUFA because of its high content of n-3 PUFA and PUFA in general (Bergé and Barnathan, 2005). Due to hypotriglyceridemic effect of PUFA (Sanz *et al.*, 1999; Newman *et al.*, 2002), apart from the aim to enrich broiler meat with n-3 PUFA, *Chlorella* sp. administration therefore may also be subjected to reduce fat content of broiler as well as to improve defense system of broiler as shown in human and mice studies (Liang *et al.*, 2004; Spolaore *et al.*, 2006).

In contrast to Korver and Klasing (1997) and Wang *et al.* (2000) who reported that the effect of dietary n-3 PUFA source on the profile of tissue FA and immune competence was dose dependent, Pulz and Gross (2004) have shown that very small amounts of the genera *Chlorella* can positively affect the physiology of animals. In the form of meal, however, Nitsan *et al.* (1999) found that the levels of algae 0.1 - 1.0% from total diet had low and inconsistent effects. Concerning the level and time of n-3 PUFA incorporation, Jaturasitha *et al.* (2009) included n-3 PUFA (tuna oil) in the diet of

pig and found that a decreased concentration of n-3 PUFA in the diet of pig (1%) fed for the entire fattening period was as effective as the larger quantity of n-3 PUFA (3%) applied in the period before slaughter. On this background, the objective of this study was therefore to evaluate the effect of *Chlorella* sp. in the form of meal administrated in different levels in the diet of broilers throughout their life upon the FA profile of the breast muscle, mucosal secretory IgA (sIgA) concentration and production index (PI) of broiler.

MATERIALS AND METHODS

Animals and diets

The experiment was conducted of which used 90 mixed-sex one day old Ross chicks, with completely randomized design of 3 treatments, with 6 repetitions and 5 broiler chicks in each pen. Before being placed on farm, the chicks were sexed, weighed and marked (using cable ties) and then allotted into 18 wire floors pens (app. 2 male and 3 female chicks each pen) for 3 different dietary treatments. They were kept in a semi closed house system. The diets were based on a single basal diet (equivalent to commercial chick starter crumbles) from d-0 to d-35. They were formulated by adding ('on top') *Chlorella* sp. (to the basal diet) as the last ingredient in the mixing process. The chemical analysis of Chlorella sp included in the experimental diet is presented in Table 1. The treatment diets were: T1: control (basal diet without enrichment of *Chlorella* sp.); T2: basal diet enriched with 5-g *Chlorella* sp./kg feed; T3: basal diet enriched with 10-g *Chlorella* sp./kg feed (see Table 2). In length of experiment, the chicks were given *ad libitum* access to the diets and water. The FA composition of the diets is described in Table 3. The *Chlorella* sp. used in this experiment was obtained from Brackishwater Aquaculture Development Centre Situbondo, East Java province, Indonesia (where it had been cultivated in an uncontrolled temperature area). It was in the form of meal.

Table 1. Chemical analysis of *Chlorella* sp. included in the experimental diets

Nutrients	Content
Crude protein (N x 6.25)	55.3
Total fat (g/100g DM)	10.3
Crude fiber (%)	5.8

*) Data were obtained from Brackishwater Aquaculture Development Centre Situbondo, East Java Province-Indonesia, based on proximate analysis

Fatty acids profile of breast muscle

One male bird from each pen was selected at d-36. After being slaughtered, breast meat (skinless) was collected from each bird and used for FA determination. The FA composition was determined based on methodology described by Bligh and Dyer (1959) with some modifications. It was performed with an HP-6890 gas chromatograph equipped with an autosampler, FID, and fused-silica capillary column (30 m x 0.25 mm x 0.2 µm film thickness). The sample (1 µl) was injected with helium as a carrier gas onto the column programmed for ramped oven temperatures (initial temperature was 110°C held for 1.0 min, then ramped at 15°C/min to 190°C and held for 5 min, then ramped at 5°C/min to 230°C and held for 5 min). Inlet and detector temperatures were held at 220°C. Peak areas and percentages were calculated using a Hewlett-Packard ChemStation software. FA methyl esters were identified by comparison with retention times of authentic standard. FA values and total lipids are reported as weight percentages.

Mucosal sIgA concentration

Inactivated ND vaccine and live vaccine against ND, IBD vaccine, live vaccine against ND were used to activate the antibodies production at d-6, d-13 and d-17 respectively. At d-36, the guts (duodenum) from those 18 slaughtered-birds were taken out and washed using water flow just to get rid of any digesta. Mucosa from the same site of the duodenum for each bird was carefully collected and placed in the sample tubes. The mucosa was collected by scraping of the gut surface. The mucosa was then stored at -80^{0}C until immunological test.

The mucosa was weighed and PBS was added (1:2, wt/wt). The samples were then vortexed and centrifuged at 4000 rpm for 10 min. The supernatant was transferred into another tube and was centrifuged again at 1600 rpm for 8 min. The last supernatant was diluted 500 times and further used for assay. Conjugate diluents pH 8.0 (0.05M Tris, 0.15M NaCl, 1% BSA, 0.05% Tween 20) was used for diluting the samples. Quantitative ELISA (Sandwich) immunoassay kits from Bethyl Laboratories (Montgomey, TX, USA) were used for measurement of chicken sIgA. The ELISA assay was, with few exceptions, performed according to the kit manual. In brief,

Table 2. Chemical Analysis of the Experimental Diets

Nutrients	T1	T2	T3
Dry matter (%)	88.5	88.3	88.3
Crude protein (N x 6.25)	20.8	20	20.8
Total fat (g/100g DM)	5.92	6.19	7.46
Crude fiber (%)	2.4	2.8	2.3
Ash content (%)	6.4	6.6	6.4
Metabolisable Energy (MJ/100kg)	1243	1189	1214

microtitre plates (96-wells) were coated with 100µl the capture antibody (anti-chicken IgA) followed by incubation at room temperature (20-25^{0}C) for 2 h. After incubation, the solution was removed and the plate was washed 3 times with wash solution pH 8.0 (0.05M Tris, 0.15M NaCl, 0.05% Tween 20). Then 200µl blocking solution was added to each well and incubated at room temperature for 30 min, the solution was removed and washed 3 times hereafter. Standards and samples (100µl) were added to each well followed by room temperature incubation for 1h and washed 3 times. The sIgA concentration was detected by incubation with HRP-conjugated goat anti-chicken IgA antibody and then incubated for 1h and washed 3 times hereafter. TMB (3,3´,5,5´-tetramethyl benzidine) was used as chromagen and 1M H_2SO_4 as stop solution. The result was monitored as OD at 450 nm. The sIgA concentration was calculated from a standard curve as suggested by the company. The final result was accounted by multiplying with the dilution factor for each sample.

Since mucosa was sometimes difficult to scrape with the same force each time, so some scraping samples somehow could contain more gut layers than other samples. To minimize the error in measuring the mucosal sIgA concentration, the sIgA concentration was then corrected by the gram of total protein contained in the mucosa. Thus mucosal sIgA concentration was presented as mg IgA per gram total protein (contained in the mucosa).

Quantification of total protein in the gut mucosa

Total protein in the gut mucosa was quantified by Auto analyser-Advia 1650, based on the method of Weichselbaum (1946) using biuret reagent. Samples used for the quantification were taken from the samples for the mucosal sIgA analysis (supernatant from the second centrifugation before the dilution step). In brief, 60 µl samples were put in the Auto analyser, 120 µl 0.9% saline was added and mixed hereafter. From the mixture, 17.5 µl was taken and reacted with 62.5 µl reagent (cupric sulfate in an alkaline solution). Protein peptide bonds interact with the cupric ions to form a purple complex was then measured as an endpoint reaction at 545 nm.

Production index (PI) of broiler

All chicks were weighed individually at d-0 and d-35, whereas at d-7, d-14, d-21 and d-28 the birds were weighed as a pen group. Feed intakes were recorded weekly for each pen, thus feed conversion ratio (FCR) could be calculated. Estimates of weekly feed intake were made by subtracting the total weekly residue weight from the total weight of feed offered for that week. Mortality was observed daily. The production index (PI) was calculated at d-35 based on the formula suggested by Lima and Nääs (2005) where PI = (daily weight gain, grams × livability, %) / (FCR x 10).

Statistical analysis

All data were presented as the mean ± the standard error of the mean. The FA composition in the breast muscle, mucosal sIgA concentration and PI of broiler were analyzed using a one-way ANOVA procedure. Effect of different levels of *Chlorella* sp. administration in the diet was analyzed. All analysis was performed by SPSS 15.0 for Windows. A p-value of less than 0.05 was considered statistically significant.

RESULTS AND DISCUSSION

Fatty acids profile of breast muscle

Chlorella sp. administration had no significant effect ($p>0.05$) on the content of saturated fatty acid (SFA), monounsaturated fatty acids (MUFA) and PUFA as well as the total fat content of the breast muscle of broiler. The content (data not shown) and ratio of n-3 to n-6 FA were also not affected by *Chlorella* sp. administration (Table 4).

It has been known that lipid content of broiler is directly affected by dietary fat level (Sanz *et al.*, 1999; Crespo and Esteve-Garcia, 2001). Peebles *et al.* (1997) show that increasing dietary fat level increases carcass fat content of broiler. In contrast to these studies, our findings show that increasing the level of fat in the diets due to *Chlorella* sp. administration did not produce an increased fat content of broiler; i.e.

increased dietary fat level statistically and numerically lowered the percentage of abdominal fat content to live body weight (BW) of broiler (data not shown) and total fat content in the breast muscle of broiler, respectively (see Table 4). This result suggests that the reduction of lipid content of broiler is strongly related to the dietary FA profile instead of dietary fat level *per se*. High level of PUFA in our experimental diets (Table 3) might produce less fat content through suppressing *de novo* FA synthesis and enhancing FA β-oxidation (Crespo and Esteve-Garcia, 2001; Ratnayake and Galli, 2009). It should also be noted that high total SFA content in the diets might also suppress *de novo* FA synthesis (Ratnayake and Galli, 2009). Coetzee and Hoffman (2002) reported that increasing the level of PUFA in the diets is also effective in reducing the level of SFA in the carcasses of chickens. This is in agreement with our findings, in which although statistically was not different, numerically the content of SFA in the breast muscle was lower in birds provided higher level of PUFA derived from *Chlorella* sp. *De novo* FA synthesis in animal systems produce only SFA and MUFA (Volpe and Vagelos, 1973). Thus, decreased lipogenesis followed by the lower level of SFA as well as MUFA (especially oleic acid; C18:1n-9) could be expected.

In animal tissues the desaturation of the *de novo* synthesized FA stops with the production of the MUFA (not PUFA). Therefore, PUFA content of the breast muscle was assumed not to be related to the lipogenesis but was more related to the dietary PUFA content, since deposition of PUFA in the tissue strongly depends on the dietary supplementation (Lo´pez-Ferrer et al., 2001; Barroeta, 2004). However, this is not in our case since PUFA content in the breast muscle did not increase following the increase of dietary PUFA. Cunnane and Anderson (1997) and Fu & Sinclair (2000) reported that PUFA in general are preferentially oxidized for metabolic purposes. Consistent to the previous discussion, we suggest that higher level of dietary PUFA in our study enhanced the rate of PUFA oxidation led to diminished tissue PUFA incorporation. Much higher level of linoleic acid (LA) compared to α-linolenic acid (ALA) in the diets might also explain the high rate of β-oxidation, particularly for ALA (Pan and Storlien, 1993). The deposition and conversion of n-3 or n-6 PUFA into their longer chain homologues (eicosapentaenoic acid EPA and docosahexaenoic acid DHA or arachidonic acid AA, respectively) are influenced by the ratio between those FAs (Ratyanake and Galli, 2009). The similar ratio between the FAs in our diets (Table 3), especially between ALA and LA following *Chlorella* sp. administration, therefore, resulted in an insignificant difference upon the ratio of n-3 to n-6 PUFA in the breast muscle. After all, it could be suggested that beside depending on the level of dietary n-3 PUFA, the effect of this dietary FA on tissue PUFA composition appears to depend on the nature of n-3 PUFA source as well, since not all dietary n-3 PUFAs are biologically equivalent.

Table 3. FA Composition (mg/100g) of the Experimental Diets

Fatty acids	T1	T2	T3
Total SFA [1]	1935.99	2017.36	2456.82
Total MUFA [2]	1897.92	1961.44	2354.55
Total n-3 PUFA [3]	137.66	142.17	174.88
Total n-6 PUFA [4]	1841.58	1957.96	2345.65
Total PUFA [5]	1979.24	2100.13	2520.53
Ratio n-3/n-6 PUFA	0.07	0.07	0.07

[1]Total SFA calculated as C14:0 + C16:0 + C18:0

[2]Total MUFA calculated as C16:1n-7+C18:1n-9+C18:1n-7

[3]Total n-3 PUFA calculated as C18:3n-3+C20:5n-3+ C22:5n-3+C22:6n-3

[4]Total n-6 PUFA calculated as C18:2n-6+C18:3n-6+ C20:3n-6+C20:4n-6+C22:5n-6

[5]Total PUFA calculated as n-3 PUFA+n-6 PUFA

Mucosal sIgA concentration

Being presented as mg sIgA per gram total protein (contained in the mucosa), it was not affected significantly (P>0.05) by *Chlorella* sp. administration (Table 4). However, it appears that increased levels of *Chlorella* sp. in the diet was numerically accompanied by the higher concentration of mucosal sIgA.

The production of sIgA is of major importance to the function of the mucosal immune systems of broiler. Like the other type of immunoglobulins, the mucosal sIgA concentration of poultry is affected by the ratio of n-3 to n-6 PUFA through eicosanoid mediated effect (Miura et al., 1998; Yang and Guo, 2006). In accordance to Spolaore *et al*. (2006) who have shown that alga as a part of animal feed could improve the immune response, and also concomitant to Yang and Guo (2006), our results suggest that increased

Table 4. FA Composition in the Breast Muscle, mg sIgA per g-total Protein Contained in the Mucosa, Production Index (PI) of Broiler at d-35 and Mortality Rate of Broiler

Dietary Treatments	Total fat (g/100g DM)	Total SFA (mg/100g DM)	Total MUFA (mg/100g DM)	Total PUFA (mg/100g DM)	Ratio of n-3 to n-6 FA	mg sIgA/g-total Protein	Production Index (PI)	Mortality rate (%)
T1	0.85±0.10	266.87±29.34	339.48±48.97	246.95±23.80	0.07±0.004	14.32 ±4.24	360.24±39.69	10.00±4.47
T2	0.78±0.10	249.55±30.51	302.27±44.53	238.50±24.18	0.07±0.002	26.84 ±7.30	373.97±51.87	10.00±6.83
T3	0.68±0.07	220.08±21.21	264.14±34.43	210.08±16.83	0.08±0.002	32.98 ±6.99	385.52±41.64	6.67±6.67
p value	*0.47*	*0.49*	*0.49*	*0.47*	*0.31*	*0.19*	*0.92*	*0.91*

levels of *Chlorella* sp. resulted in numerically higher concentration of mucosal sIgA. Since the ratio of n-3 to n-6 PUFA in our diets was similar, we hypothesized that the overall effect of *Chlorella* sp. on the mucosal sIgA production could not be accounted for solely on the basis of eicosanoid mediated effect. Mice and chicken studies show that cytokines are crucial for sIgA production (Kramer et al., 1995; Yang and Guo, 2006; Yang et al., 2006). Therefore it is most likely that the effect of *Chlorella* sp. was mediated through cytokines (Yoshida *et al.*, 2001; He *et al.*, 2007). Nuclear transcription factors-κB (NF-κB) is involved in regulating, synthesis and expression of cytokines (Calder, 1998), where its activity is stimulated by n-6 PUFA (Camandola *et al.*, 1996). The n-3 PUFA have also been identified to potentiate the activity of cytokines in chickens, particularly a group of interleukin (IL-2, IL-4 and IL-6) (Yang and Guo, 2006; Yang *et al.*, 2006). Taken together, both n-3 and n-6 PUFA contained in the diets therefore were assumed to promote the production of cytokines leading to higher production of mucosal sIgA.

Production index (PI) of broiler at d-35

Following the records of daily weight gain, FCR and mortality (livability) rate, the calculation of PI was performed at d-35. Administration of *Chlorella* sp. in the diets of broiler had no significant effect (P> 0.05) on daily weight gain, FCR and mortality rate of broiler (data not shown). Since none of significant differences upon the factors influencing the PI could be observed, insignificant differences of PI (Table 4) among the dietary groups could be expected (Lima and Nääs, 2005). The weight gain and FCR of the birds were not affected significantly by dietary PUFA derived from *Chlorella* sp. These were concomitant to Newman *et al.* (2002) and Chen and Chiang (2005), respectively.

Mortality rate following feeding different levels of *Chlorella* sp. was not significantly different (P>0.05) (see Table 4). These findings were consistent with Mirghelenj *et al.* (2009). Based the observation, the major cause of mortality during rearing was a heat stress accompanied by colibacillosis incidences. It was because birds were kept in the semi closed house system where the temperature could not be controlled rigidly. However, there was a tendency that broilers fed high level of *Chlorella* sp. had a lower mortality rate compared to those fed control diet. Those chickens fed higher level of *Chlorella* sp. probably had better immune response reflected by the higher mucosal sIgA concentration.

CONCLUSION

The level and ratio between n-3 to n-6 PUFA as well as the nature of dietary PUFA source determine tissue PUFA composition. Beside through eicosanoid, *Chlorella* sp. may affect mucosal sIgA production through cytokines mediated effect. Although *Chlorella* sp. administration does not improve the production index of broiler, this treatment may produce broiler meat with lower fat content.

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